Journal of Medical Virology 51:210–213 (1997)

High Interferon Alpha Levels in Placenta, Maternal, and Cord Blood Suggest a Protective Effect Against Intrauterine Herpes Simplex Virus Infection

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Interferons (IFN) are produced by the placenta during pregnancy, and they can be detected in the maternal and fetal blood. Although the antiviral potential of IFNs is well established, it remains unclear whether the IFNs associated with pregnancy can prevent transplacental spread of viral infection. The present study was undertaken in order to determine the possible protective effect of placentally produced IFN-α on fetal acquisition of herpes simplex virus (HSV). Nine mothers with a known history of genital HSV infection were studied. In five cases IFN-α was detected in the placenta, maternal, and fetal blood, whereas in three cases IFN-α could not be detected. In the remaining case, IFN-α was found only in the maternal blood. As corroborated by the serological evidence of early HSV infection in the cord blood, the single case of vertical HSV transmission was observed in the group of IFN nonproducers. Furthermore, virus transmission did not occur in cases where IFN-α was present in the placenta and simultaneously in the maternal and fetal circulations. Thus, the present data indicate that high levels of IFN during pregnancy may protect the fetus from acquiring a possibly fatal intrauterine HSV infection. ***J. Med. Virol. 51:210–213, 1997.***

KEY WORDS: vertical transmission; fetal infection; transplacental

INTRODUCTION

In North America, neonatal herpes simplex virus (HSV) infection occurs with an approximate frequency of 1 in every 3,500 deliveries [Whitley, 1993]. Since the infection readily disseminates, it is associated with significant morbidity and mortality [Whitley et al., 1991]. Intrauterine infection encompasses less than 5% of the neonatal HSV infections [Whitley and Arvin, 1995]. However, children exposed to intrauterine infection are more likely to present with congenital malformations such as microcephaly, microophthalmia, or hydraencephaly compared to children with perinatal infection [Hutto et al., 1987; Whitley and Arvin, 1995]. The pathogenesis of intrauterine infection is not completely understood, but it is likely that the virus spreads across the placenta, with subsequent transmission to the fetus [Witzleben and Driscoll, 1965; Robb et al., 1986a,b]. Furthermore, recent data show that the villous trophoblast in vitro supports replication of HSV [Nørskov-Lauritsen et al., 1992].

The body of evidence indicates that the two most important factors affecting intrauterine acquisition of virus are 1) primary vs. recurrent maternal HSV infections and 2) the protective effect of placentally transferred virus-specific IgG [Whitley, 1993]. Limited information is available on the effect of nonspecific antiviral mechanisms on the protection of the fetus against the infection. The present study was conducted to shed more light on the possible participation of placentally produced interferons (IFN) in the prevention of vertical HSV transmission.

MATERIALS AND METHODS

Collection of Biological Material

After informed consent, mothers with a known history of genital HSV infection attending a major obstetric clinic in Aarhus, Denmark, were recruited (the collection of biological material was approved by the local ethical committee). All together, nine placentas with linked specimens of clotted maternal and cord blood

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Accepted 2 October 1996

TABLE I. Serological Analysis of Maternal and Cord Blood Pairs From the Cohort of HSV-Infected Mothers*

Patient	Serum	Total Ig (g/l)			HSV Ig ratio[a]			CRP (mg/ml)	IFN-α (IU/ml)
		IgG	IgM	IgA	IgG	IgM	IgA		
1	Mother	7.46	1.78	1.92	**9.4**	0.89	0.62	23	1,960[b]
1	Cord	12.54	<0.05	<0.06	**11**	0.67	0.55	<5	910[b]
2	Mother	9.42	0.92	1.18	**14**	0.55	1.18	8	<5
2	Cord	14.02	0.09	<0.06	**16**	0.47	0.49	<5	<5
3	Mother	8.01	2.50	2.20	**5.8**	0.69	0.48	7	541[b]
3	Cord	12.34	0.12	<0.06	**5.4**	0.58	0.42	<5	185[b]
4	Mother	7.57	2.21	2.35	**16**	1.01	1.03	<5	324
4	Cord	9.87	0.08	<0.06	**16**	1.41	1.27	<5	<5
5	Mother	8.21	2.70	2.53	**3.0**	1.37	0.46	15	<5
5	Cord	9.99	0.08	<0.06	**4.2**	0.58	0.46	<5	<5
6	Mother	7.63	1.57	1.54	**9.4**	0.67	0.63	14	2,100[b]
6	Cord	13.71	<0.05	<0.06	**7.4**	0.75	0.91	<5	2,190[b]
7	Mother	7.16	1.28	1.46	**4.9**	0.61	0.48	19	1,880[b]
7	Cord	10.76	<0.05	<0.06	**6.3**	0.52	0.47	<5	1,670[b]
8	Mother	9.25	1.80	2.15	**7.5**	0.58	0.44	14	76[b]
8	Cord	13.18	0.20	<0.06	**11**	0.70	0.57	<5	117[b]
9	Mother	9.33	1.47	0.94	**2.9**	0.78	0.50	11	<5
9	Cord	11.17	0.11	<0.06	**4.9**	0.76	0.54	<5	<5

*IgG, IgM, IgA, and CRP were quantitated by nephelometry, and HSV-specific IgG, IgM, and IgA antibodies were determined by ELISA. The IFN levels were expressed relatively to an international standard using ELISA. The sera positive for anti-HSV Ig are shown in bold face.
[a]The serum with a ratio >1 was considered positive for HSV-Ig.
[b]IFN concurrently present in the placenta.

were obtained within 8 hr after delivery. The serum was harvested and stored at −20°C until use. The placental biopsies were taken from the fetal and maternal sides, as well as from the center of each placenta and fixed in buffered 10% formalin.

Serology

The total IgG, IgA, and IgM immunoglobulins and the C-reactive protein (CRP) were measured in all sera by nephelometry (Behring, Germany). To ensure that the cord blood was free from maternal contamination, the placentally produced human chorionic gonadotropin (hCG) was measured in cord and maternal serum. hCG is present in maternal serum at a high level (12.9 IU/ml), in contrast to cord blood (0.038 IU/ml) [Chen et al., 1993]. In this study, high levels were only demonstrated in the maternal serum, thus the introduction of maternal serum to the cord serum skewing the results from the cord blood could be excluded. In addition, a ratio of class A and class M Ig in maternal and cord blood with that of specific antibodies of the identical class in maternal and cord blood was compared to confirm that the antibodies found in the cord blood were unequivocally of fetal origin.

The presence of HSV-specific IgG, IgA, and IgM antibodies was determined semiquantitatively by enzyme-linked immunosorbent assay (ELISA; Kreatech Biotechnology B.V., The Netherlands). The content of specific antibodies in the samples tested was expressed in relation to the average of the absorbency values of the cut-off control samples provided by the manufacturer. Consequently, all samples with values above 1 were considered as positive. Furthermore, all serum samples were subjected to testing for the presence of IFN-α by ELISA (BioSource Int., USA). Using a standard curve, the concentration of IFN-α in the samples was expressed in IU/ml.

Immunohistology

A two-step indirect immunoperoxidase technique was applied to the paraffin-embedded placental sections. Microwave treatment was used to reveal the concealed epitope sites, and the endogenous peroxidase activity was quenched using 3% H_2O_2. All sections were tested with monoclonal antibodies (mAb) against HSV type-1 (clone LP13, Serotec, UK), HSV type-2 (clone BO19, Serotec), and IFN-α (AB-20-050, Bio-Source, USA). Unspecific mouse IgG was used as a negative control (X0931, DAKO, Denmark). Adjacent sections were stained with mAb against cytokeratin (M717, DAKO) to determine whether the HSV detected or IFN-α was found in cells of trophoblast origin. After addition of the secondary rabbit polyclonal anti-mouse antibody labeled with peroxidase (P161, DAKO), the antibody deposits were visualized by employing diaminobenzidine as a chromogen. Finally, the preparations were counterstained using Mayer's hematoxylin.

RESULTS

In maternal sera, the total Ig were found to be within the physiological range: IgG 7.16–9.42, IgM 0.92–2.70, and IgA 0.94–2.53 g/l (Table I). However, in the cord sera, the range of total IgG, when compared with maternal blood, was higher, ranging from 9.36 to 14.07 g/l, whereas the levels of IgM and IgA were much lower or undetectable. Furthermore, CRP was not found in any of the cord blood samples, whereas levels from 7 to 23 mg/l were detected in the maternal group, except for one patient (no. 4) in whom the level was below the sensitivity limit.

All mothers and children were positive for HSV-IgG,

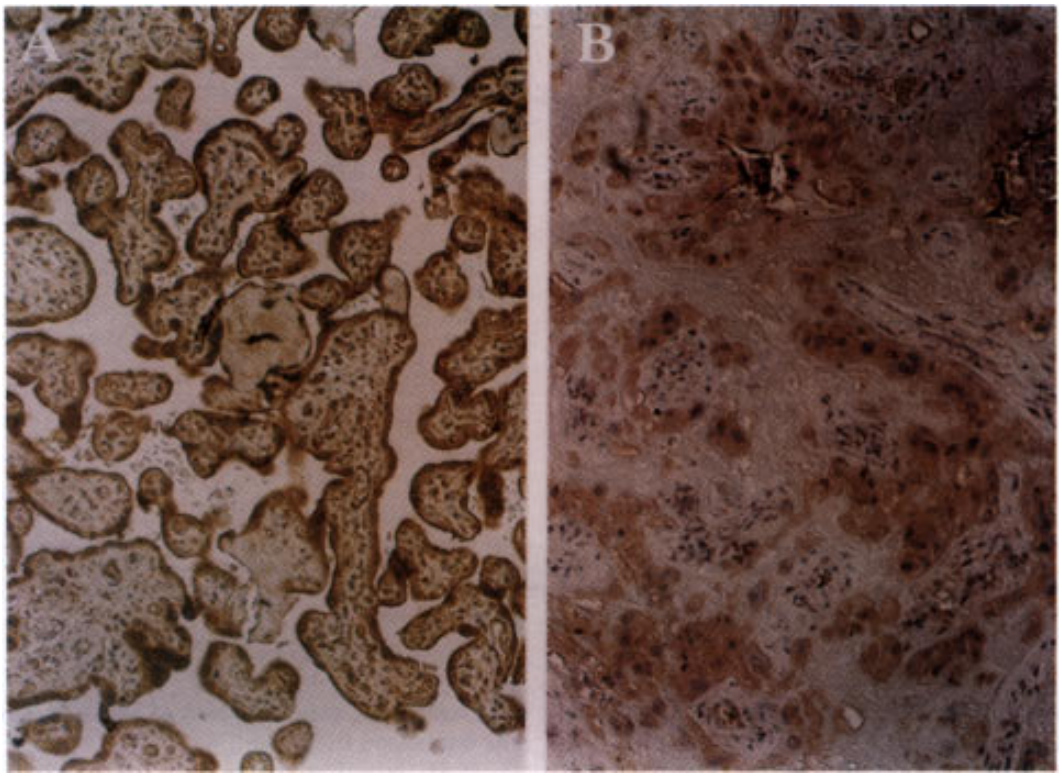

Fig. 1. Indirect immunoperoxidase detection of IFN-α in the placenta of a HSV-infected mother. The IFN-α was detected in both villous **(A)** and extravillous **(B)** trophoblast cells and the micrograph is from a representative placenta. The sections were counterstained with Mayer's hematoxylin. ×200.

and except in case no. 3 the level of IgG in the cord serum was equal to or higher than that in the corresponding maternal serum. In case no. 4, both maternal and cord blood contained HSV-specific IgM and IgA, but IFN-α was detected exclusively in the maternal serum. Furthermore, in this case CRP was not detected in the serum. In two other cases (no. 2 and 5), the mothers had HSV-specific IgA or IgM in the serum. However, in these patients an IFN-α response was not found in the cord or in the maternal sera. High IFN-α levels concurrent in maternal and cord blood were found in five patients (no. 1, 3, 6, 7, and 8). Moreover, in all these cases IFN-α was also detected by immunohistology in the placental tissue (see below). The remaining case (no. 9) was negative for IFN-α, HSV-IgA, and HSV-IgM.

The immunohistochemical examination of the placental sections did not detect HSV-1 or HSV-2-specific antigens (data not shown). Furthermore, none of the placentas presented evidence of villitis. IFN-α in the sections was confined to the trophoblast, notably the villous cytotrophoblast and syncytiotrophoblast layer (Fig. 1A) and the extravillous cytotrophoblast (Fig. 1B). Interestingly, IFN-α was found only in the biopsies from those five placentas, where high IFN levels were detected simultaneously in the mother and cord blood (Table I). Thus, in case no. 4, the placenta was negative immunohistochemically for IFN-α, even though IFN-α was detected in the maternal serum.

DISCUSSION

In the present study, a correlation between IFN-α detected in trophoblast cells and its presence in cord and maternal serum was demonstrated. Similar results were reported previously with IFN-α. However, in that investigation IFN-β could not be detected in the placenta [Ebbesen et al., 1995]. The finding of low and high IFN-α producers in the population studied is in accord with previous reports showing that type I IFNs are produced endogenously during normal human pregnancy [Lebon et al., 1982; Duc-Goiran et al., 1985; Paulesu et al., 1991], and that low/high producers of IFN exist [Bocci et al., 1985]. Even though the observation of IFN-α in the trophoblast could be due to both production and take up, the body of evidence shows that IFNs are produced by trophoblast cells [Bocci et al., 1985; Tóth et al., 1990; Aboagye-Mathiesen et al., 1991; Whaley et al., 1994]. There is some controversy concerning the specific trophoblast cell-producing IFN-α in the placenta. In the present study it was detected in the villous cytotrophoblast and syncytiotrophoblast layer and in the extravillous trophoblast. Previous studies detected IFN-α in the villous cytotrophoblast without any gestational variation [Howatson et al., 1988], in the villous syncytiotrophoblast with a decrease towards term [Paulesu et al., 1991], in all trophoblast subpopulations, notably the extravillous tro-

phoblast [Whaley et al., 1994], and in accordance with the present data [Ebbesen et al., 1995].

CRP is an acute phase protein produced in the liver during a number of infectious and noninfectious diseases. Bacterial infections are associated with a higher value than are viral infections [Chasisilwattana and Monif, 1989]. Based on a previous study showing that the CRP value immediately after delivery was 12 ± 6 mg/l [Kelemen et al. 1986], the maternal values in this study are within the normal range. CRP is detectable in the fetus before it acquires immunological capability [Ash, 1933], and it is not transmitted across the placental barrier. The CRP values obtained here do not indicate an ongoing infection.

Even though the fetal immune system is considered immature, the fetus is capable of synthesizing both HSV-specific IgM and IgA when encountering an intrauterine infection [Kahlon and Whitley, 1988]. In one case (no. 4), both the fetus and mother were positive for HSV-specific IgM and IgA, suggesting that HSV had been present in both circulations. Here, IFN-α was only detected in the maternal serum. Since the placenta was negative for IFN-α and there was no evidence of infection of the placenta, it suggests that the IFN-α detected in the maternal serum was induced from a nonplacental maternal focus. Thus, it seems that high levels of IFN-α, if present in the maternal and fetal circulation and at the same time also in placenta, may be effective in preventing HSV transmission to the fetus. The marked antiviral activity of in vitro-induced trophoblast IFNs [Tòth et al., 1990] indicates that the pregnancy-associated IFN-α plausibly exerts a similar antiviral effect during infection in vivo.

Moreover, an additional line of evidence for the putative inhibitory effect of IFNs on HSV transplacental passage is provided by the recent observation based on a study of the relationship of IFN-α and the inhibition of vertical human immunodeficiency virus (HIV) transmission [Zachar et al., 1996]. Although limited in size, this initial study lends support to the notion that IFNs during pregnancy may provide protection against at least some congenital viral infections, and may potentially prove beneficial in cases where the risk of infection is increased intrapartum.

ACKNOWLEDGMENTS

This work was supported by a grant from the Danish Cancer Society. The biological material was kindly provided by the Department of Gynecology and Obstetrics, University Hospital of Aarhus, Denmark; Head, Prof. N.J. Secher. The technical assistance of Birgitte Tønning and Jette Jaugesen is appreciated.

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